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Decreased soluble IFN-β receptor (sIFNAR2) in multiple sclerosis patients: A potential serum diagnostic biomarker

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Abstract

Background: The soluble isoform of the interferon-β (IFN-β) receptor (sIFNAR2) could modulate the activity of both endogenous and systemically administered IFN-β. Previously, we described lower serum sIFNAR2 levels in untreated multiple sclerosis (MS) than in healthy controls (HCs).

Objective: To assess sIFNAR2 levels in a new cohort of MS patients and HCs, as well as in patients with clinically isolated syndrome (CIS) and with other inflammatory neurological disorders (OIND) and to assess its ability as a diagnostic biomarker.

Methods: The cross-sectional study included 148 MS (84 treatment naive and 64 treated), 87 CIS, 42 OIND, and 96 HCs. Longitudinal study included 94 MS pretreatment and after 1 year of therapy with IFN-β, glatiramer acetate (GA), or natalizumab. sIFNAR2 serum levels were measured by a quantitative ELISA developed and validated in our laboratory.

Results: Naive MS and CIS patients showed significantly lower sIFNAR2 levels than HCs and OIND patients. The sensitivity and specificity to discriminate between MS and OIND, for a sIFNAR2 cutoff value of 122.02 ng/mL, were 70.1%, and 79.4%, respectively. sIFNAR2 increased significantly in IFN-β-treated patients during the first year of therapy in contrast to GA- and natalizumab-treated patients who showed non-significant changes.

Conclusion: The results suggest that sIFNAR2 could be a potential diagnostic biomarker for MS.

Keywords: Multiple sclerosis, soluble interferon beta receptor, diagnosis, biomarker

Introduction

Multiple sclerosis (MS) is a chronic autoimmune disorder characterized by inflammation of the central nervous system (CNS), demyelination, and axonal damage.[1] IFN-β is a key cytokine in MS as it contributes to the maintenance of the anti-inflammatory status of the immune system[2] and is one of the most widely used therapies for MS patients, even if the mechanism by which it exerts its beneficial effects is not completely understood. Its biological activity is mediated through interaction with the interferon (IFN) α or β cell surface receptor (IFNAR) which is composed of two subunits: IFNAR1 and IFNAR2.[3] IFNAR2 binds IFN-β, and IFNAR1 is required to form and stabilize the IFN-β-receptor high-affinity complex. The interaction of IFN-β with IFNAR activates the intracellular signaling pathway that involves the JAK-STAT protein family and that concludes with the activation of the genes containing the interferon-

stimulated response element (ISRE).[4]

Soluble cytokine receptors provide mechanisms to modulate the cytokine responses.[5] As they have the ability to bind to their ligands, they can act as carrier proteins to protect the cytokine from proteolysis, improve the stability, or decrease clearance.[6–8] Moreover, solu- ble receptors can act as antagonists competing with the transmembrane form of the receptor[9] or as agonists through the trans-signaling mechanism, as in the case of the soluble receptor of interleukin-6 (IL-6).[10]
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An alternative splicing of the RNA encoding the cytokine receptor and/or cleavage of membrane receptor are the two major mechanisms for the generation of soluble receptors.[5] Focusing on IFNAR2, three isoforms result from the alternative processing of the human IFNAR2 gene.[11] The short form is a nonfunctional transmembrane protein with a truncated cytoplasmic domain; the long form is a full-length transmembrane protein, which comprises the functional receptor together with IFNAR1;[12] and the third transcript encodes the soluble form of the receptor: sIFNAR2 (IFNAR2.3 or IFNAR2a). The protein from the latter transcript lacks the transmembrane and cytoplasmic domains[13] and can be detected in body fluids.[14] These sIFNAR2 transcripts have been found to be expressed at 10-fold higher levels than the transmembrane IFNAR2 transcripts in most tissues.[15] Although the role of sIFNAR2 has not yet been explained, a recombinant murine sIFNAR2 and the extracellular domain of IFNAR2 can both bind IFN-β with nanomolar affinity,[16] and a modulatory function of sIFNAR2 has been postulated, in which low concentrations would increase the bioactivity of IFN-β, whereas high concentrations might decrease its effect.[17]

Previous studies, two of them performed by our group, have evaluated the gene expression of the two subunits of IFN-β receptor (IFNAR1 and IFNAR2) in MS patients.[18–21] However, unlike the transmembrane forms, the soluble isoform of the IFN-β receptor (sIFNAR2) has hardly been studied in MS, and even less at protein level, and its role has been overlooked despite the important evidence indicating that it could modulate the activity of both endogenous and systemically administered IFN-β.[15]

Previously, a recombinant human sIFNAR2 protein was cloned, expressed, and purified in our laboratory; after which, we developed and validated an ELISA for its quantification in human serum. The validated ELISA was used to determine sIFNAR2 concentration in serum samples from untreated MS patients and healthy controls (HCs), showing that sIFNAR2 levels in MS patients were significantly lower than those in HCs.[22] Consequently, our main objective was to conduct a descriptive study of sIFNAR2 in serum to evaluate its potential use as a biomarker that could contribute to the diagnosis of MS.

Material and methods

Subjects

Human serum samples were obtained from 84 treatment naïve and 108 treated patients with MS defined according to the revised McDonald criteria[23] recruited from the Malaga Regional University Hospital (Malaga, Spain). All MS patients were relapsing–remitting. The samples were always obtained during clinical remission, and the patients had not received corticosteroids in the 3 months prior to blood sampling. Treatment-naïve patients had never received any disease modifying therapy. For the cross-sectional study, 84 treatment-naïve and 64 IFN-β-treated patients were included. As HCs, 96 unrelated age-matched healthy individuals were selected. Additionally, 42 patients with other inflammatory neurological disorders (OIND) were included: 24 from Ramón y Cajal Hospital (Madrid, Spain) and 18 from the Málaga Regional University Hospital. OIND included 11 patients with Guillain-Barré syndrome, 9 with limbic encephalitis, 8 with recurrent myelitis, 4 inflammatory cranial nerve palsy, 2 with sarcoidosis, 3 with systemic lupus erythematous, 2 with isolated CNS vasculitis, and 1 with each of the following pathologies: Behçet's disease, Tolosa-Hunt syndrome, and autoimmune ganglionopathy. A total of 87 untreated patients with clinically isolated syndrome (CIS) from the Centre d'Esclerosi Múltiple de Catalunya were also included.

To evaluate the effect of the treatment on the sIFNAR2 levels, a 1-year follow-up study was carried out including 50 of the 64 IFN-β-treated patients included in the cross-sectional study, 26 patients treated with glatiramer acetate (GA), and 18 patients treated with natalizumab.

All subjects who participated in the study gave informed consent, and the protocols were approved by the institutional ethical committee (Comité de Ética de la Investigación Málaga Nordeste). Demographic characteristics of the subjects are shown in Table 1.

Sample collection

For treatment-naïve patients, 5 mL of peripheral blood was collected before starting the therapy. For treated patients, samples were obtained before treatment and after 1 year of therapy with IFN-β, GA, or natalizumab. The time elapsed after the last administering of treatment was at least 24 hours before sampling. Cerebrospinal fluid (CSF) samples were collected by lumbar puncture. All the samples from MS patients and HCs were processed following standard procedures and frozen immediately after reception by the Málaga Hospital-IBIMA Biobank, as part of Andalusian Public Health System Biobank.

Table 1. Demographic and clinical characteristics of the participants.

Cross-sectional study						Longitudinal follow-up study		
	Control group	Naive MS patients	CIS	OIND	IFN-β-treated MS patients	IFN-β-treated MS patients	GA-treated MS patients	Natalizumab-treated MS patients
N	96	84	87	42	64	50	26	18
Female/male	65/31	49/35	58/29	26/16	45/19	31/19	18/8	13/5
Age (years)[a]	40.10 ±12.26	42.37 ±13.73	33.14 ±7.72	52.20 ±14.52	43.97 ±10.08	37.75 ±11.41	38.00 ±19.16	39.57 ±11.48
Disease duration[a] (years)	–	5.9 ± 7.03	–	–	13.2 ± 8.41	4.5 ± 5.8	7.5 ± 10.70	11.11 ± 8.80
EDSS score at baseline	–	1 (0–2)	–	–	1 (0–1.5)	1 (0–1.5)	1 (1–2.5)	1 (1–4)
Treatment duration	–	–	–	–	6.25 ± 1.49	1 ± 0.1	1 (±0.09)	1±0.1
Previously treated with ODMD	–	–	–	–	0/64	0/50	0/26	5/13

MS: multiple sclerosis; CIS: clinically isolated syndrome; OIND: other inflammatory neurological disorders; IFN: interferon; GA: glatiramer acetate; EDSS: Expanded Disability Status Scale; ODMD: other disease modifying drugs.
[a]Age and duration of disease are presented as mean ±standard deviation. EDSS scores are expressed as median (interquartile range).

Quantification of soluble IFNAR2 in serum by ELISA

Quantification of sIFNAR2 was performed with an ELISA developed and validated in our laboratory,[22] whose parameters of validation, accuracy, and precision met the acceptance criteria for immunoassays of protein biomarkers.[24]

Briefly, recombinant sIFNAR2 protein was cloned using the prokaryotic expression system pEcoli-Cterm 6xHN linear. DH5α Competent Cells™ were transformed with the plasmid. After plasmid purification and once the nucleotide sequence and the correct reading frame had been verified, BL21(DE3) bacteria were transformed to produce the recombinant protein sIFNAR2, which was purified on high-capacity Ni^{+2}-iminodiacetic acid resin columns and detected by Western blot and was also identified by matrix-assisted laser desorption or ionization time-of-flight mass spectrometry (MALDI-TOF), as described in Orpez T, 2015.

This sIFNAR2 recombinant protein was used as standard curve in the ELISA. For this, 96-well plates were coated with 0.2-µg rabbit polyclonal anti-human IFNAR2 antibody (Abnova) overnight. The wells were washed four times and blocked with casein blocking buffer (2 hours, room temperature (RT)). Then, 50 µL of each point of the standard curve or serum samples (diluted half) was added to the wells in duplicate and incubated for 1 hour. Mouse polyclonal anti-human IFNAR2 antibody (1 µg/mL in assay buffer; Abnova) was added and incubated (1 hour, RT). After three washes, 50-µL horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG (H + L) adsorbed against human immunoglobulins was added to each well and incubated (1 hour, RT). After three additional washes, 100 µL/well 3,3′,5,5′-tetramethylbenzidine (TMB) One Component HRP Microwell Substrate was added and incubated (10–15 minutes in the dark). Color development was terminated by adding 50 µL/well 1 N H_2SO_4. Optical density (OD) was measured at 450 nm in a VersaMax microplate reader.

Sample analysis

sIFNAR2 concentration in human serum samples was evaluated in duplicate. Each assay included a standard curve with seven concentrations (ranging from 3.9 to 250 ng/mL), two quality controls, and a negative control. The sIFNAR2 concentration was determined by OD interpolation from the samples and controls in the standard curve. The calibration curve was established using a four-parameter curve fitting model (SoftMax Pro, Molecular Devices).

Sample measurement was considered acceptable if the intra-assay coefficient of variation (CV) was <10% and the inter-assay CV was <20%.[24] With the assays included in this study, the average of the intra-assay CV was 3.6% and average of the inter-assay CV was 13%.

Statistical analysis

The data were analyzed with SPSS 15.0 (SPSS, Chicago, IL). Quantitative variables were reported as

mean ± standard deviation (SD) or median and interquartile range (IR), where appropriate. As a non-normal distribution was established in the Kolmogorov–Smirnov test, non-parametric Kruskal–Wallis and Mann–Whitney *U* test (two independent variables) were used to compare sIFNAR2 levels between groups. A $p < 0.05$ was considered statistically significant. To correct for multiple testing, the Bonferroni correction was used to calculate the cutoff value for significance level (0.05/5 = 0.01). A Wilcoxon test was used to compare sIFNAR2 levels between pretreatment samples and samples after 1 year of therapy with IFN-β, GA, or natalizumab. Finally, a receiver operating characteristic (ROC) curve analysis was performed to define the optimal cutoff values of sIFNAR2. Positive and negative predictive values were calculated using the recommended cutoff value.

Results

Evaluation of sIFNAR2 in serum

We have previously published that sIFNAR2 levels in MS patients (median (IR): 71.67 (39.67–128.18 ng/mL)) were significantly lower than those in HCs (median (IR): 134.3 (76.10–179.21 ng/mL); $p < 0.00001$).[22] To evaluate whether sIFNAR2 could be a potential biomarker for MS, we have now evaluated sIFNAR2 levels in an independent cohort of treatment-naïve MS patients, a group of patients with CIS, a group of patients with OIND, and a different cohort of HCs.

In this second cohort, naïve MS patients showed significantly lower levels of sIFNAR2 than HCs ($p = 6.64 \times 10^{-10}$) and OIND patients ($p = 1.32 \times 10^{-7}$). Besides, CIS patients also had significantly lower levels of sIFNAR2 when compared with HCs ($p = 2.31 \times 10^{-20}$) and OIND patients ($p = 2.55 \times 10^{-15}$), but there were no differences with naïve MS patients ($p = 0.070$). The heterogeneous group of OIND showed no significant differences with HC ($p = 0.764$), and its levels were significantly increased when compared with IFN-β-treated patients ($p = 0.013$).

However, IFN-β-treated MS patients displayed significantly higher levels of sIFNAR2 than naïve MS patients ($p = 0.001$) and CIS ($p = 2.39 \times 10^{-10}$) and were significantly lower than HC ($p = 0.003$).

There were no differences in sIFNAR2 concentration according to gender nor correlation with age or Expanded Disability Status Scale (EDSS) in none of the study groups included. The values of sIFNAR2 obtained for the different study groups are summarized in Table 2 and Figure 1.

Table 2. Serum sIFNAR2 concentration in the different groups of the study.

	N	sIFNAR2 (ng/mL)
		Median (P_{25}–P_{75})
Healthy controls	96	153.64 (115.29–228.33)
Naive MS patients	84	70.43 (37.37–138.68)
CIS patients	87	56.21 (40.54–89.92)
OIND patients	42	150.70 (126.28–175.89)
IFN-β-treated patients	64	125.42 (89.46–163.33)

MS: multiple sclerosis; CIS: clinically isolated syndrome; OIND: other inflammatory neurological disorders; IFN: interferon.

Regarding CIS patients, the cohort included 47 who converted to clinically definite multiple sclerosis (CDMS) and 38 patients who did not convert (CIS). The follow-up period between the samples collection of CIS patients and MS conversion was 5.0 years (SD: 3.5 years). There were no differences in sIFNAR2 levels between CDMS (68.3 ± 48.8) and CIS (68.0 ± 44.5; $p = 0.853$).

Going back to the naive patients, to verify the stability of the sIFNAR2 levels over time, five naive patients of whom we had two samples collected were analyzed with a time window of a month before starting any pharmacological therapy. There were no significant differences in sIFNAR2 levels between the two time points (time 1: mean (SD), 112.69 (±61.9); time 2: mean (SD), 93.26 (±74.72); $p = 0.225$)).

Evaluation of sIFNAR2 in CSF

To evaluate the levels of sIFNAR2, we analyzed CSF from 26 naïve MS patients (with positive oligoclonal bands) and a control group of 22 patients (with normal CSF analysis and negative oligoclonal bands) with a diagnosis of benign intracranial hypertension. The results showed that CSF sIFNAR2 levels in MS patients were significantly lower than in the control group (77.35 (53.10–94.50) vs 101.1 (81.48–138.04); $p = 0.007$; Figure 2).

Evaluation of soluble IFNAR2 as a diagnostic biomarker for MS

The differences observed between naïve MS patients suggested that serum levels of sIFNAR2 could be a potential diagnostic biomarker for MS. Accordingly, an

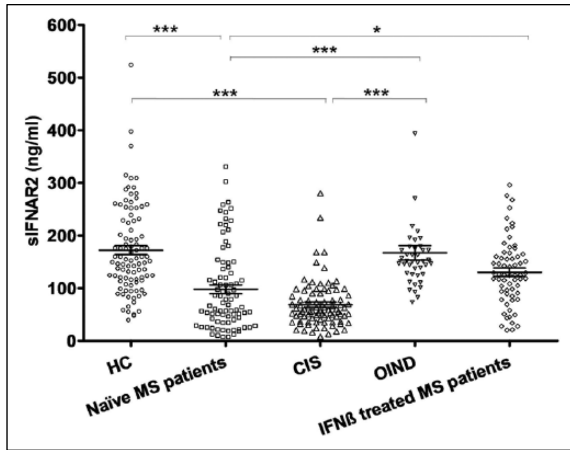

Figure 1. Serum sIFNAR2 levels. Serum sIFNAR2 levels were determined in naïve MS patients, CIS, OIND, HC, and IFN-β-treated patients by ELISA, as described in section "Material and methods." Significant *p* values are shown with asterisk. Serum sIFNAR2 levels are significantly decreased in non-treated MS patients and CIS compared to HCs and patients with OIND.

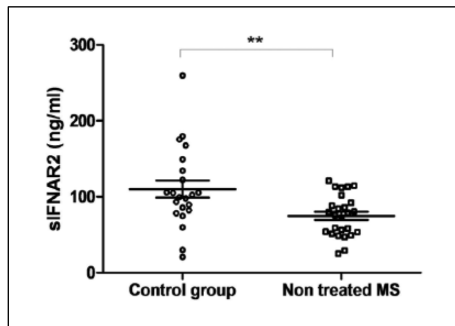

Figure 2. CSF sIFNAR2 levels. CSF sIFNAR2 levels were determined in non-treated MS patients and in a control group by ELISA, as described in section "Material and methods." Significant *p* values are shown with asterisk. CSF sIFNAR2 levels decreased significantly in non-treated MS patients.

ROC curve analysis was carried out to evaluate the accuracy and discriminating capacity of the test. First, the ROC curve was constructed including 84 naïve MS patients and 133 non-MS patients (OIND + HC), and an area under the curve (AUC) of 0.785 (95% confidence interval = 0.715–0.855; $p < 0.0001$) was obtained. The optimal cutoff value to discriminate between MS patients and non-MS patients was 120.13 ng/mL of sIFNAR2, which resulted in a sensitivity of 71.0%, a specificity of 76.3%, a positive predictive value of 60.7%, and a negative predictive value of 79.0%.

After this, the ROC curve was constructed including 84 naïve MS patients and 96 HCs, and an AUC of 0.791 (95% confidence interval = 0.722–0.861; $p < 0.0001$) was obtained. The optimal cutoff value to discriminate between MS patients and HCs was also 120.13 ng/mL of sIFNAR2, which resulted in a sensitivity of 72.6%, a specificity of 77%, a positive predictive value of 74.4%, and a negative predictive value of 75.3%.

Finally, to analyze the capacity to discriminate MS patients from OIND patients, an ROC curve was constructed including 84 naïve MS patients and 42 OIND patients, and an AUC of 0.771 (95% confidence interval = 0.690–0.852; $p < 0.0001$) was obtained. In this case, the optimal cutoff value to discriminate between MS patients and OIND patients was 122.02 ng/mL of sIFNAR2, which resulted in a sensitivity of 70.11%, a specificity of 79.4%, a positive predictive value of 88.4%, and a negative predictive value of 64.38%.

Changes in sIFNAR2 levels during treatments

To evaluate the dynamic changes in sIFNAR2 levels during treatments, 94 treated patients were included. Serum samples were taken at baseline before the onset of therapy and after 12 months of treatment. From these patients, 50 received one of the commercially available IFN-β molecules, 26 GA, and 18 natalizumab. sIFNAR2 determinations at baseline and after treatment were performed in the same ELISA plate to minimize the inter-assay variation.

sIFNAR2 concentrations increased over a period of 1 year compared with the levels at baseline in 36 of the 50 (72%) IFN-β-treated patients, these differences being significant ($p = 0.001$).

However, in the group of GA- and natalizumab-treated patients, 38% and 33% of the patients, respectively, increased sIFNAR2 levels over a period of 1 year, but the differences before and after treatment were not significant, as observed in Figure 3.

Discussion

Soluble receptors of cytokines normally participate in the control of cytokine activity. Detectable levels of these receptors can be measured in healthy individuals and in some diseases, and some of these soluble receptors have been shown to circulate in high molar excess when compared with their cytokines, as is the case for human soluble IL-7 receptor.[25] Their ability of binding to the cytokine has prompted interest in their use as markers of disease activity or even as therapeutic agents.[26]

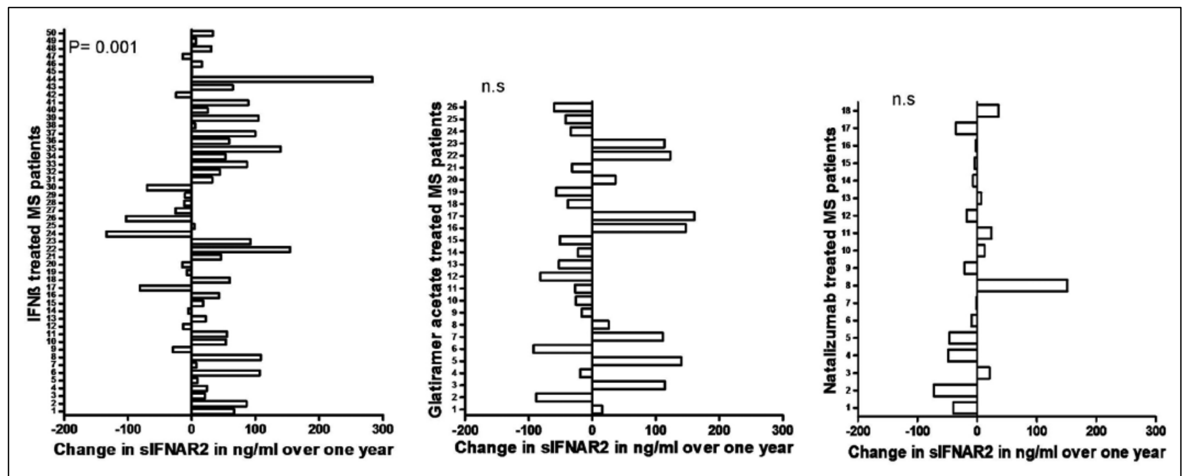

Figure 3. Serum sIFNAR2 levels during 1 year of treatment. sIFNAR2 levels were determined in serum from patients treated with IFN-β, glatiramer acetate, or natalizumab at baseline and 12 months after treatments onset, by ELISA. The bars represent the difference in sIFNAR2 levels between these two time points. sIFNAR2 levels increased significantly over a period of 1 year in IFN-β-treated patients; however, there were no significant differences in patients treated with glatiramer acetate or natalizumab.

The IFN-β-soluble receptor has been evaluated in different diseases such as hepatitis C virus (HCV),[27] acquired immune deficiency syndrome (AIDS),[28] and neoplasms.[28] However, sIFNAR2, which is able to modulate the activity of both endogenous and systemically administered IFN-β,[15,17,29] remains hardly studied in autoimmune diseases, including MS, despite the evidence that IFN-β plays an important role in the pathogenesis of these diseases.[2]

In general, biomarkers have become important tools in clinical practice; however, validated methods must be used in their assessment to improve the quality of the data and their potential application. In our previous work, a recombinant protein, analogous to human sIFNAR2, was cloned, expressed, and purified. This protein was used as a standard in the development and validation of a suitable ELISA for the quantification of sIFNAR2 in human serum. We applied this validated assay to quantify serum sIFNAR2 in MS patients and HCs, showing that non-treated MS patients had significantly lower circulating sIFNAR2 levels than HCs, highlighting the importance that sIFNAR2 could have in MS pathogenesis and its potential use as a diagnostic biomarker.[22]

Based on these results, we have carried out a replication study including an independent cohort of naïve MS patients, CIS, HCs, and OIND patients. In this study, we corroborated the existence of lower levels of circulating sIFNAR2 in naïve MS patients than in HCs. We also measured sIFNAR2 in CSF, and its distribution is similar to that found in serum, with lower levels in naïve MS patients. Being strict with the detection method, although our ELISA is validated for measuring sIFNAR2 in serum, additional validation would be necessary for measurement of sIFNAR2 in CSF. However, the ELISA worked well with CSF samples, and the sIFNAR2 mean in CSF from MS patients was similar to that found in serum.

The stability over time of this biomarker has been assessed by measuring serum levels of IFNAR2 in untreated MS patients at different time points before starting any pharmacological therapy, showing that sIFNAR2 levels were stable in these patients whose samples were taken within a time window of a month.

In the past, several studies reported increased levels of serum sIFNAR2 in patients with chronic viral infections such as HCV[27] or HIV,[28] systemic lupus erythematosus,[28] or adenocarcinoma.[30] Most of these studies speculated an inhibitory function of sIFNAR2 on IFN-β activity although definitive evidence of its action in vivo was not detailed. However, a recent study aimed to investigate the effects of sIFNAR2 overexpression in vivo concluded that circulating IFN-β receptor clearly potentiates signal transduction of type I IFNs, being an important agonist of endogenous type I IFN actions in pathophysiological processes, and that increased levels of sIFNAR2 are likely to facilitate IFN signaling.[29] Consequently, the lower sIFNAR2 circulating levels observed in naïve MS patients may contribute to a suboptimal activation of the endogenous IFN-β signaling pathway. Something similar could be happening in patients

with a CIS, in which sIFNAR2 circulating levels were similar to levels found in naïve patients.

The differences observed between naïve MS patients and HC or patients with other neurological diseases suggest that sIFNAR2 could be a potential diagnostic biomarker for MS, so we evaluated the relevance of our results in the clinical context using the ROC analysis. Our results suggest that serum sIFNAR2 measurement may be a useful and easy-performing biomarker contributing to MS diagnosis since it has a sensitivity of 72.6% and a specificity of 77.0%, with the established cutoff, to discriminate from HC. Regarding the ability to discriminate from OIND patients, sIFNAR2 showed very similar values with a sensitivity of 70.11% and a specificity of 79.4%. Taking into account that we are analyzing one univariate indicator, sIFNAR2 levels show a significant discriminatory ability to distinguish between MS patients and HCs or OIND patients; this ability might improve by combining with additional markers that should be investigated further, as has been described with other soluble receptors addressed as potential biomarkers.[31]

However, the data obtained from the CIS cohort suggest that sIFNAR2 would not be a biomarker associated with the conversion from CIS to MS since there were no differences between CDMS patients and patients who remained as CIS.

In relation with the therapy, the only previous work that evaluates sIFNAR2 levels in IFN-β-treated patients showed elevated sIFNAR2 levels in stable IFN-β-treated MS patients when compared with active MS patients.[32] In our cross-sectional study, patients undergoing IFN-β treatment presented elevated levels of sIFNAR2 when compared with naïve patients. Furthermore, we evaluated the dynamic change in sIFNAR2 levels during the treatment. In a follow-up study, we have shown that IFN-β-treated patients increased sIFNAR2 levels over a period of 1 year, in contrast to GA- and natalizumab-treated patients, whose sIFNAR2 levels were mainly constant. As it has previously been explained, sIFNAR2 is an important regulator not only of endogenous IFN-β but also of the systemically administered type I IFN.[15,29] But, reciprocally, our data suggest that IFN-β treatment also increases sIFNAR2 levels significantly. This increase could be mediated by the action of proteases which would release the extracellular portion of IFNAR2.2 by shedding as a consequence of over stimulation of the IFNAR receptor by exogenous IFN-β,[33] although further studies are needed to evaluate this hypothesis.

To our knowledge, this is one of the first studies to reveal differential sIFNAR2 levels between naïve MS patients and HCs and OIND patients, suggesting a role for sIFNAR2 as a biomarker for MS. In consequence, it could be added to the panel of other potential laboratory diagnostic biomarkers described in MS kappa free light chains[34] or chitinase 3–like 1,[35] among others, with the great advantage of being assessed in serum instead of CSF.

However, considering that sIFNAR2 has recently been found to be an important agonist of the endogenous IFN actions,[29] we cannot explain so far whether the lower levels of sIFNAR2 could somehow contribute to the pathogenesis of MS or otherwise be a consequence of the underlying pathogenic process, so that the role of sIFNAR2 will have to be investigated further.

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Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: Ó. Fernández received honoraria as consultant to advisory boards, and as chairman or lecturer in meetings, and has participated in clinical trials and other research projects promoted by Almirall, Actelion, Allergan Bayer-Schering, Biogen Idec, Novartis, Merck-Serono, Roche, and Teva. L.M. Villar received travel funding and/or speaker honoraria from Biogen, Merck-Serono, Novartis, Genzyme, Teva, Bayer, and Roche. M. Comabella has received compensation for consulting services and speaking honoraria from Bayer Schering, Biogen Idec, Genzyme, Merck-Serono, Novartis, Sanofi, and Teva. X. Montalban served on the scientific advisory board for Novartis, Teva, Merck, Biogen, Bayer, Almirall, Actelion, Genzyme, Octapharma, Receptos, Roche, Sanofi, and Trophos and received travel funding from Novartis, Teva, Merck, Biogen, Bayer, Almirall, Acetelion, Genzyme, Octapharma, Receptos, Roche, Sanofi, and Trophos. J.C. Alvarez-Cermeño is on the scientific advisory board for Biogen Idec, Novartis, Genzyme, Roche, and Merck-Serono; received speaker honoraria from Biogen Idec, Novartis, Genzyme, and Merck-Serono; and received research support from Biogen Idec, Novartis, and Merck-Serono. All the other authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in this manuscript apart from those disclosed.

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